THE INDUCTION OF THE ENZYMES OF FATTY ACID DEGRADATION IN ESCHERICHIA COLI

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Received August 15, 1967

Although the pathways for the degradation and the synthesis of saturated and unsaturated fatty acids have been worked out in detail (see reviews by Lynen, 1955; Vagelos, 1964; and Stoffel, 1966), very little is known about the regulation and the genetics of the enzymes involved in the various reactions. We have therefore started to isolate mutants which affect the fatty acid metabolism of E. coli K12. Mutants which have lesions in fatty acid synthesis can be obtained as fatty acid auxotrophs. A mutant requiring unsaturated fatty acids has been described in a recent report by Silbert and Vagelos (1967). Similar mutants have been found by us (P. Overath, unpublished). Mutants which are blocked in fatty acid catabolism are unable to use fatty acids as sole carbon source. In the course of these studies we found that the enzymes of fatty acid degradation are inducible in E. coli. This paper describes the induction of five enzymes of fatty acid breakdown by oleate. Reactions 1 to 3 are part of the usual β-oxydation cycle and reactions 4 and 5 are required for the complete breakdown of unsaturated fatty acids. The enzymes catalyzing the following reactions are shown to be inducible in the presence of oleate:

1) crotonyl-CoA + H_2O $\rightleftharpoons$ (crotonase*) L-3-hydroxybutyryl-CoA

2) L-3-hydroxybutyryl-CoA + NAD $\rightleftharpoons$ (HOADH) acetacetyl-CoA + $NADH_2$

3) acetacetyl-CoA + CoA $\rightleftharpoons$ (thiolase) 2 acetyl-CoA

4) dodecen-3cis-oyl-CoA $\rightleftharpoons$ (isomerase) dodecen-2trans-oyl-CoA

5) D-3-hydroxylauryl-CoA $\rightleftharpoons$ (epimerase) L-3-hydroxylauryl-CoA

Methods

Organisms. The prototrophic strain E. coli K12Ymel (obtained from U. Henning) was used. Acl0 is a mutant of this strain lacking pyruvate dehydrogenase and was isolated by Henning et al. (1964). Mutants in which the degradation of oleic acid is blocked will be called old (from oleic acid degradation). Such mutants can be readily isolated after mutagenesis with N-methyl-N'-nitro-N-nitroso-guanidine (Adelberg et al., 1965) by plating first on minimal glucose plates and replica plating on minimal oleate plates.

Growth conditions. Mineral solution M9 and tryptone medium LT were prepared as described by Lengeler (1966). 0.5% glucose, 0.5% Na-succinate or 0.5% glycerol were added as carbon source to M9. For media containing fatty acids M9 was mixed with 1% polyethyleneglycolmonolaurylether (Brij 35, Serva Entwicklungslabor, Heidelberg, Germany) in a ratio of 6 : 4. To this solution 0.1% Na-oleate or other fatty acids were added. Brij 35 cannot serve as a carbon source. Solid media were prepared by adding 1.5% agar.

Preparation of extracts. The cells were grown aerobically in the various media to late logarithmic phase (optical density at 420 mμ of 1.5 - 2). 1.2 g washed cells (wet weight) were suspended in 4 ml 0.1 M phosphate buffer pH 6.8 and sonified for 2 min. at 0-5 °C with a Branson Sonifier. The extracts were centrifuged for 15 min. at 105 000 x g. The supernatants were directly used for the enzyme determinations.

Enzyme assays. Crotonase: (Stern and DelCampillo, 1955; Decker, 1962a). 175 μmoles diethanolamine-buffer pH 9.5, 2 μmoles EDTA, 0.3 μmoles NAD, 20 μg HOADH (Boehringer and Soehne, Mann-

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*Abbreviations: crotonase = L-3-hydroxyacyl-CoA-hydro-lyase (E.C.4.2.1.17); acyl-CoA synthetase = acid: CoA ligase (AMP) (E.C.6.2.1.3); HOADH = 3-hydroxy-acyl-CoA-dehydrogenase = L-3-hydroxyacyl-CoA: NAD oxydoreductase (E.C.1.1.1.35); isomerase = Δ^{3cis}-Δ^{2trans}-enoyl-CoA-isomerase (E.C.5.3.3.?); epimerase = 3-hydroxy-acyl-CoA-3-epimerase (E.C.5.1.2.3.)

heim, Germany) and 0.1 µmole crotonyl-CoA (Decker, 1955). V = 1ml, T = 24 °C, λ = 334 mµ. HOADH: (Lynen et al., 1952; Decker, 1962b). 50 µmoles phosphate-buffer pH 6.8, 2 µmoles EDTA, 0.25 µmoles $NADH_2$, 1 mg serum albumin , 0.25 µmoles acetacetyl-CoA (Decker, 1955). V = 1 ml, T = 24 °C, λ =366 mµ. Thiolase: (Lynen and Ochoa, 1953). 200 µmoles tris-buffer pH 8.1, 5 µmoles $MgCl_2$, 0.2 µmoles CoA, 5 µmoles Na-thioglycolate, 0.085 µmoles acetacetyl-CoA. V = 1 ml, T = 24 °C, λ = 313 mµ. A molar extinction coefficient of 12.2 x 10^3 (1 x $mole^{-1}$ x cm^{-1}) was used for the calculation of the specific activity (Gehring, 1964). Isomerase: (Stoffel et al., 1964b). This enzyme was assayed after the 105 000 x g supernatant had been heated for 1 min. at 70 °C and centrifuged at 20 000 x g for 15 min. 300 µmoles tris-buffer pH 9.2, 4 µmoles EDTA, 0.6 µmoles NAD, 10 µg HOADH, 4 µg crotonase (Stern et al., 1956), 0.085 µmoles dodecen-3-cis-oyl-CoA (Stoffel et al., 1964a). V = 2 ml, T = 23 °C, λ = 340 mµ. Epimerase: (Stoffel et al., 1964b). Same conditions as for the isomerase only that 0.1 µmole D-3-hydroxylauryl-CoA (Stoffel et al., 1964a) served as substrate and crotonase was omitted.

Results

Preliminary experiments showed that ^{14}C-fatty acids (oleic acid, palmitic acid, caprylic acid) are rapidly incorporated into the lipids of E. coli. In accord with Silbert and Vagelos (1967) it was found that oleic acid can serve as the sole carbon source for this organism. The same is true for myristic, palmitic, palmitoleic, cis-vaccenic, trans-vaccenic, linoleic and linolenic acid. In a mutant, Acl0, auxotrophic for acetate, the acetate requirement can be replaced by the above fatty acids. Thus E. coli must have the enzymes for the breakdown of these compounds. E. coli cannot grow on butyric, caproic, caprylic and capric acid and grows only poorly on lauric acid. Since it is known from the work of Goldfine and Bloch (1961) that fatty acids of medium chain length can be elongated to long-chain fatty acids in E. coli, this organism must have the enzyme to convert them to the respective thiolesters. The inability of the C_4-C_{10}-fatty acids to serve as carbon source might therefore be due to their inability to induce the enzymes of fatty acid degradation.

The table shows the specific activities of the five enzymes listed above in extracts of cells grown with or without oleic acid in the presence of other carbon sources. All enzymes have the highest specific activity in cells grown on oleate as sole carbon source. When grown in the absence of oleic acid on other carbon sources, all enzymes show levels of 1% or less compared to oleate grown cells. The enzymes are readily induced by oleic acid in the presence of succinate or LT. The induction shown in the

Table. Induction of the enzymes of fatty acid degradation in *E. coli*

strain	carbon source	crotonase sp.a.	%	HOADH sp.a.	%	thiolase sp.a.	%	isomerase sp.a.	%	epimerase sp.a.	%
K12Ymel	oleate	9.3	100	11.9	100	0.44	100	34.3	100	4.9	100
"	LT	0.05	0.5	0.14	1.2	0.004	0.8	<0.02	<0.1	<0.02	<0.4
"	LT + oleate	2.5	27	4.2	35	0.18	41	9.6	28	2.0	41
"	LT + Brij	0.54	5.8	0.91	7.6	0.033	7.5	<0.02	<0.1	<0.02	<0.4
"	glucose	0.03	0.3	0.05	0.4	0.002	0.5	0.045	0.13	<0.02	<0.4
"	glucose + oleate	0.03	0.3	0.02	0.2	0.002	0.5	0.08	0.23	<0.02	<0.4
"	succinate	0.35	3.8	0.38	3.2	0.017	3.9	0.1	0.3	0.073	1.5
"	succinate + oleate	2.0	21	3.0	25	0.13	30	8.6	25	1.9	39
"	glycerol	0.12	1.3	0.21	1.8	0.057	1.3	0.043	0.13	<0.02	<0.4
"	glycerol + oleate	0.26	2.8	0.49	4.1	0.017	3.9	0.68	2.0	0.16	3.3
old 5	LT	<0.005	<0.05	<0.02	<0.2	<0.002	<0.5	<0.02	<0.1	<0.02	<0.4
"	LT + oleate	0.005	0.05	0.03	0.3	0.002	0.5	<0.02	<0.1	<0.02	<0.4
old 30	LT	0.047	0.5	0.12	1.0	<0.002	<0.5	<0.02	<0.1	<0.02	<0.4
"	LT + oleate	4.2	45	10.6	91	0.002	0.5	5.0	15	1.2	25

sp.a. = specific activity: in μmoles/mg/min for crotonase, HOADH and thiolase, in mμmoles/mg/min for isomerase and epimerase

control (LT + Brij) is probably due to small amounts of fatty acids in the crude preparation of this detergent. The absence of any induction in the presence of glucose is remarkable, whereas glycerol shows an intermediate behaviour. This is another example of the "glucose effect" which is well known especially for enzymes of sugar metabolism (Magasanik, 1961). Since the proportion of the enzymes crotonase, HOADH and thiolase is roughly the same under all conditions, it seems likely that they are under coordinate control and perhaps comprise an operon. The relative values for the isomerase and the epimerase are also similar to the other enzymes, however it cannot be decided at present if the deviations are significant. The E. coli-isomerase shows properties (heat stability, resistance to SH-reagents) very similar to the mammalian enzyme (Stoffel, Caesar and Ditzer, 1964b). Preliminary experiments indicate the presence of the acyl-CoA-synthetase in oleate grown E. coli. The enzyme has not been detected in this organism before (Kornberg and Pricer, 1953). It is predominantly bound to the membrane fraction (spec. act. 46 mμmoles/mg/min.).

From about 40 old - mutants isolated so far the properties of two are included in the table. old 5 has barely detectable levels of crotonase, HOADH and thiolase, the other two enzymes were too low to be measured. This is the behaviour expected for "polar" mutations found in many operons. old 30 essentially lacks thiolase but contains nearly wild-type levels of HOADH, crotonase, isomerase and epimerase. The presence of isomerase and epimerase in this strain excludes the possibility that these enzymes are induced by an intermediate of the breakdown of oleic acid and is in accord with the assumption that they are also induced by oleic acid or a derivative thereof.

Acknowledgement: This work was supported by a grant (5R05 TW 00237) from the United States Public Health Service to one of us (P.O.)

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